

## WHAT IS CLAIMED IS:

*Sub C29*

1. A method for producing an enantiomerically pure  $\alpha$ -substituted carboxylic acid, said method comprising contacting an aldehyde or ketone with a cyanide containing compound and an ammonia-containing compound or an ammonium salt or an amine, and stereoselectively hydrolyzing the resulting amino nitrile or cyanohydrin intermediate with a nitrilase or a polypeptide having nitrilase activity, wherein the nitrilase is sufficiently active to perform the hydrolysis in the presence of the reaction components, under conditions and for a time sufficient to produce the carboxylic acid.

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2. The method according to claim 1, wherein said enantiomerically pure  $\alpha$ -substituted carboxylic acid has the following structure:

$$\begin{array}{c}
 \text{HOOC} \\
 \diagup \quad \diagdown \\
 \text{C*} \\
 \diagdown \quad \diagup \\
 \text{R}_1 \quad \text{R}_2
 \end{array}
 \quad \text{E}$$

wherein:

15  $\text{R}_1$  and  $\text{R}_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $\text{R}_1$  and  $\text{R}_2$  are linked to cooperate to form a functional cyclic moiety and

20  $\text{E}$  is  $-\text{N}(\text{R}_x)_2$  or  $-\text{OH}$ , wherein each  $\text{R}_x$  is -H or lower alkyl.

3. The method according to claim 2, wherein said enantiomerically pure  $\alpha$ -substituted carboxylic acid is an  $\alpha$ -amino acid.

25 4. The method according to claim 3, wherein at least one of  $\text{R}_1$  and  $\text{R}_2$  is substituted or unsubstituted aryl.

5. The method according to claim 4, wherein said enantiomerically pure  $\alpha$ -amino acid is D-phenylalanine, D-phenylglycine, or L-methylphenylglycine.

6. The method according to claim 3, wherein said enantiomerically pure  $\alpha$ -amino acid bears a substituted or unsubstituted alkyl side chain.

5 7. The method according to claim 6, wherein said enantiomerically pure  $\alpha$ -amino acid is L-tert-leucine, D-alanine, or D-hydroxynorleucine.

8. The method according to claim 2, wherein said enantiomerically pure  $\alpha$ -substituted carboxylic acid is an  $\alpha$ -hydroxy acid.

10 9. The method according to claim 8, wherein at least one of  $R_1$  and  $R_2$  is substituted or unsubstituted aryl.

15 10. The method according to claim 10, wherein said enantiomerically pure  $\alpha$ -hydroxy acid is (S)-cyclohexylmandelic acid, mandelic acid or 2-chloro mandelic acid.

11. The method according to claim 1, wherein the cyanide is a metal cyanide or a gaseous cyanide.

20 12. The method according to claim 11, wherein the cyanide is an alkali cyanide.

13. The method according to claim 11, wherein the metal cyanide is sodium cyanide.

25 14. The method according to claim 1, wherein the ammonium salt has the formula  $NH_2(R)_2^+X^-$ , wherein each R is independently  $-H$  or lower alkyl, and X is a counter ion.

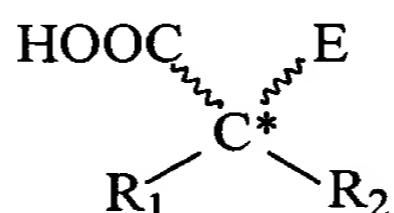
15. The method according to claim 14, wherein X is a halide.

16. The method according to claim 15, wherein the halide is  $Cl^-$ .

30 17. The method according to claim 16, wherein the ammonium salt is  $NH_4^+Cl^-$ .

18. An enantiomerically pure  $\alpha$ -substituted carboxylic acid produced by a process comprising combining an aldehyde or ketone with a metal cyanide, ammonia or an ammonium salt, and a nitrilase, under conditions and for a time sufficient to produce the 5 carboxylic acid.

19. The enantiomerically pure  $\alpha$ -substituted carboxylic acid according to claim 18, having the structure:



10 wherein:

15  $\text{R}_1$  and  $\text{R}_2$  are each independently -H, substituted or unsubstituted alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, heterocyclic, wherein said substituents are lower alkyl, hydroxy, alkoxy, mercapto, cycloalkyl, heterocyclic, aryl, heteroaryl, aryloxy, or halogen or optionally  $\text{R}_1$  and  $\text{R}_2$  are linked to cooperate to form a functional cyclic moiety, and  
E is  $-\text{N}(\text{R}_x)_2$  or  $-\text{OH}$ , wherein each  $\text{R}_x$  is -H or lower alkyl.

20. The enantiomerically pure  $\alpha$ -substituted carboxylic acid according to claim 19, wherein the carboxylic acid is an  $\alpha$ -amino acid.

25 20 21. The enantiomerically pure  $\alpha$ -substituted carboxylic acid according to claim 18, wherein the carboxylic acid is an  $\alpha$ -hydroxy acid.

22. The method according to claim 1, wherein the nitrilase has an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4.

23. The method according to claim 1, wherein the nitrilase is encoded by a nucleic acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:3.

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24. The method according to claim 1, wherein the nitrilase has an amino acid sequence at least 70% identical to the amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 and has nitrilase activity.

5 / 25. A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 and sequences having at least 70% identity thereto and having nitrilase activity.

10 / 26. An isolated nucleic acid sequence encoding an amino acid sequence as set forth in SEQ ID NO:2 or SEQ ID NO:4 and sequences having at least 70% identity thereto and having nitrilase activity, and fragments thereof that hybridize to the nucleic acid sequence.

15 / 27. An isolated nucleic acid sequence as set forth in SEQ ID NO:1.

20 / 28. An isolated nucleic acid sequence as set forth in SEQ ID NO:3.

29. A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:2.

30. A substantially purified polypeptide having an amino acid sequence as set forth in SEQ ID NO:4.

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